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# CARDIAC MYXOMA THE GREAT IMITATORS: COMPREHENSIVE HISTOPATHOLOGICAL AND MOLECULAR APPROACH

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## ABSTRACT

Cardiac myxomas are rare benign and slowly proliferating neoplasms of uncertain histogenesis with heterogeneous histomorphology and variable and sometimes clinically quite malignant pathological manifestations. Majority of cardiac myxoma occur sporadically while a relatively small proportion of diagnosed cases develop as a part of Carney complex syndrome with established familial pattern of inheritance. Although histologically indistinguishable these two forms of cardiac myxoma exhibit distinct cytogenetic make-up and apparent pathological differences important for their clinical presentation and prognosis.

Additional problem is presented with secondary lesions with more aggressive histology and significantly faster cell proliferation suggesting their successive malignant alteration. Surgical resection of cardiac myxoma is currently the only treatment of choice. However, to avoid potentially hazardous operating procedures and possible postoperative complications and to prevent recurrence of the neoplastic lesions it is necessary to develop alternative approaches and identify a possible drug targets for their successful pharmacological treatment. Due to the rarity of the disease, a small number of cases in one institution and lack of comprehensive experimental data particularly concerning the cases of metastatic dissemination and secondary lesions with malignant nature, a comprehensive multi-institutional approach is required for better understanding of their molecular pathology and illumination of key molecular, genetic as well as epigenetic markers and regulatory pathways responsible for their development. In this article we provide comprehensive pathohistological, molecular and cytogenetic overview of sporadic cardiac myxoma cases restating the major hypothesis concerning their histogenesis and emphasizing potential approaches for their further reexamination.

## **INTRODUCTION**

Cardiac myxoma (CM) represents the most prevalent type of primary cardiac tumors in adults, constituting up to 50-85% of all benign lesions [1, 2, 3 and 4]. Contrary, during fetal development, infancy and childhood, CM makes only 15% of cardiac tumors with most of them found in older children [5, 6, and 7].

CMs are benign, slowly proliferating neoplasms that do not infiltrate into the myocardium and usually do not form metastatic lesions [1, 2, 3 and 4].

Due to their strategic localization and inherent pathohistological characteristics, they are clinically considered as quite malignant entities with very serious consequences for morbidity and mortality of affected patients [1 and 8].

Estimated incidence of CM is approximately 0.5-1 cases per  $10^6$  individuals per year, with apparent preponderance (3:1) of female patients [9 and 10].

Although usually diagnosed between the fourth and sixth decade of life, CM may be encountered in any age group, though rarely among children and with extremely rare occurrence during foetal and neonatal periods of life [6, 7 and 11].

The nomenclature of CMs is based on the cardiac chamber in which they arise (atrial vs. ventricular), the side of the heart affected (left vs. right-sided) and the exact position of their attachment within the given cardiac chamber (posterior or anterior wall, interatrial septum or atrial appendage). Their name derives from the prevalent myxoid extracellular matrix rich in glycoproteins and proteoglycans [2].

The majority of CMs (60-80%) are diagnosed within the left atrium, arising mainly from the interatrial septum of the fossae ovalis border [2, 3 and 6]. They may also originate from anywhere within the atrium including the posterior and anterior endocardial tissue and appendage [12].

About 15-28% cases of CM are diagnosed in the right atrium, 8% in the right, and 3-4% in the left ventricle. Ventricular cases of CM are mainly found in women and children. A small proportion of CMs (1.6-8.5%) are biatrial, and approximately 1.6% has multifocal origin [2, 3, 5 and 12].

There are also rare reports of CM originating from anterior and posterior mitral leaflets, pulmonary artery and vein, aortic valves, inferior vena cava and superior part of interatrial septum [12, 13, 14, 15 and 16].

Majority of CM (90%) occur sporadically, while only 5-10% have established familial pattern of inheritance [17, 18, 19 and 20].

Hereditary form of CM occurs as a part of Carney complex (CNC) syndrome, characterized with variable phenotypic expression but nearly complete penetrance.

CNC is an autosomal-dominant disease in which cardiac and extracardiac myxoma [skin, breast] arise in settings of spotty skin pigmentation [blue nevi, lentiginose, ephelides, cafe-au-lait spots], endocrine tumors and overactivity (pituitary adenoma with acromegaly or gigantism, primary pigmented nodular adrenocortical disease [PPNAD], thyroid tumors] and other neoplastic lesions [testicular large-cell calcifying Sertoli-cell tumors [LCCSCT], psammomatous melanotic schwannoma [PMS]) [21].

Although sporadic and hereditary types of CM are histologically quite indistinguishable there are some subtle but clinically still apparent differences in their presentation and prognosis.

Sporadic cases frequently arise as isolated and solitary left atrial tumors in middle age female patients, whereas familial myxoma can occur in any cardiac chambers (quite often as multicentric neoplasia), usually at an earlier period of life and with no apparent gender preferences. Furthermore, patients with CNC more often develop recurrent tumors despite adequate surgical resection of the initial lesion [22].

### **Clinical presentation**

CM may stimulate enormously wide spectrum of potentially misleading cardiac and noncardiac symptoms and signs, therefore completely deserving the infamy of the "*great imitators*" in clinical medicine [2].

Clinical presentation depends primarily on their location (left or right cardiac chamber), size and mobility and to a lesser degree on their overall morphology [10, 23, 24, 25 and 26].

Some patients (10-15%) show no clinically visible signs, particularly in the case of small neoplasms (< 40 mm) and the tumor is diagnosed entirely by chance during routine examinations for other pathological conditions or at autopsy [1 and 8].

Contrary, in some clinically advanced patients, due to the strategic location, large size and inherent fragmentation CM may precipitate acute heart failure and sudden death [27].

Patients with CM generally present with one or more symptoms of classical triad of: hemodynamic derangement due to the intracardiac obstruction, signs of systemic (peripheral or cerebral) or pulmonary embolisation and/or nonspecific constitutional symptoms [1, 2, 3 and 8].

Intracardiac obstruction with congestive heart failure is the prevailing cause of acute presenting symptoms of CM that provokes their initial diagnosis, while the neurological and other symptoms of systemic and pulmonary embolism are slightly less common.

### ***Intracardiac obstruction***

Intracardiac obstruction is initially observed in 50% of patients but may reveal itself later, at any time during the disease progression, encompassing finally up to 70% of all affected patients [26 and 28].

Hemodynamic derangements commonly give rise to symptoms of left (dyspnea, paroxysmal nocturnal dyspnea, orthopnea, pulmonary and peripheral edema, pulmonary hypertension) or right-sided (peripheral edema, ascites, hepatomegaly, other symptoms of venous hypertension) heart failure [2, 28, 29, 30, 31 and 32].

The severity of symptoms is characteristically progressive and depends primarily on obstruction intensity (tumor size), and in some cases, postural changes of afflicted patients.

The predominant abnormality is the obstruction of heart valve orifices often mimicking clinical signs of mitral or tricuspid valve stenosis.

Obstruction occurs more commonly with large pedunculated tumors with soft, easily deformable tissue. Occasionally, CM may even cause complete temporary occlusion of corresponding valve orifice, resulting in dizziness, intermittent positional syncope, or sudden death [2, 3 and 27].

In some patients frequent back-and-forth motions of deformable tumor tissue between originating and adjacent cardiac chamber ("*wrecking ball*" effect) may interfere with proper closure of valve orifice causing mitral or tricuspid valve regurgitation and/or damage to their supporting structures (chordae tendine) with concomitant development of signs of mitral or tricuspid valve insufficiency [33].

Mitral valve stenosis represents the most common problem of differential diagnosis associated with the left atrial myxoma [2].

Occasionally, CM and mitral valve stenosis may occur as separate clinical entities concomitantly present in the same patient, which further complicates the emerging clinical picture and postpones timely and comprehensive diagnosis of existing disease/s [34 and 35].

Right atrial myxomas often mimic the symptoms of tricuspid valve stenosis or insufficiency but they may also disclose clinical signs of constrictive pericarditis and pulmonary hypertension [29, 36 and 37].

Ventricular myxomas are more often sessile and less frequently responsible for intracardiac obstruction [2 and 3]. In the cases when they are sufficiently large and/or strategically located in subaortic or subpulmonary regions they may cause obstruction of blood outflow mimicking the symptoms of pulmonary or aortic valve stenosis [38, 39, 40 and 41].

Obstruction of blood flow may also be caused by rare cases of CM arising directly in the valve tissue, therefore leading to symptoms of stenosis, regurgitation and other signs of valvular insufficiency.

### ***Embolism***

Embolic manifestations have been observed in 30-50% of CM patients. They are caused by tissue fragmentation, detachment of tumor as a whole and/or dissemination of overlaying thrombi or foci of existing vegetation from tumor surface.



Due to the prevailing left-sided location of CM, systemic embolisms (cerebral and peripheral) are most frequently encountered. Primarily in cerebral and retinal arteries followed by arteries of the lower extremities, visceral, renal and coronary arteries, and sometimes even in the abdominal aorta [42, 43, and 44].

More than 50% of embolic events caused by CM affect the central nervous system (CNS) and retinal arteries, resulting in intracranial and extracranial vascular obstructions, eyesight disorders, cerebral infarction, seizure and brain necrosis or intracranial aneurysms, hemiparesis, aphasia, and progressive dementia [45, 46, 47, 48, 49 and 50].

Due to quite common embolisation of CNS some authors suggest careful histopathological evaluations of all CNS emboli to differentially exclude CM as their possible cause.

Lower extremities are the next anatomic sites frequently targeted by embolic material from left-sided myxoma [51, 52 and 53].

In these settings they are occasionally misdiagnosed as peripheral vasculitis (collagen vascular disease) with unknown etiology [54]. This further emphasizes the importance of histological evaluation of all embolectomy specimens [55].

Although the embolisation of cardiac vasculature is rarely recorded, sometimes it may represent the very first clinical sign of CM resulting with occlusion and angina or fatal myocardial infarction [56, 57 and 58].

Right-sided CM rarely provokes embolic events (10%). In those cases they may effectuate obstruction of pulmonary artery, resulting with pulmonary hypertension or even fatal fulminant pulmonary obstruction [2 and 42].

### **Constitutional manifestation**

Constitutional manifestations are present in almost 90% of CM patients [1, 2 and 3]. They usually occur independently of tumor size and location and commonly include physical

weakness, lethargy, fatigue, loss of appetite, anorexia, recent and progressive decrease in body weight and persistent and unexplained low-grade fever.

Myxoma may also effectuate development of arthralgia, myalgia, facial edema, hyperhidrosis and nocturnal haemoptysis (caused by edema and pulmonary embolization), deformed digits (clubbed/drumstick fingers), and non-specific cutaneous lesions (erythematous rash, petechiae, Raynauds phenomenon).

They are accompanied by abnormal laboratory findings such as chronic anemia (normocromatic, hypercromatic, hemolytic), and elevated inflammatory markers (elevated white blood cells, polycythemia /erythrocytosis/ and elevated erythrocyte sedimentation rate, increased level of serum C-reactive proteins and elevated immunoglobulin levels.

Less common are leukocytosis, thrombocytopenia (disturbances of clotting mechanism such as disseminated intravascular coagulation), cyanosis and presence of antinuclear and rarely antiphospholipid autoantibodies [1, 2, 3 and 25].

Nonspecific constitutional an extracardiac symptoms, signs, and complications, may also present the initial manifestations contributing to clinical misinterpretation or delay in diagnosis of CM.

Laboratory abnormalities may be misinterpreted as signs of infection (infective endocarditis, rheumatic heart disease), immunological disorders (e.g. rheumatoid arthritis, vasculitis, collagen vascular disease, and autoimmune disease) and occult malignant diseases [25, 54, 59, 60 and 61].

The etiologies of constitutional manifestation are not fully understood. They may emerge as nonspecific consequences of embolization (facial edema, myalgia, arthralgia, nocturnal haemoptysis), erythropoietin production by some atrial myxoma (erythrocytosis), mechanical destruction of cells by abnormal blood flow across the tumor surface (thrombocytopenia, hemolytic anemia- particularly associated with calcified tumors) or

autocrine production of cytokines such as IL6 and IL8 (inflammatory and autoimmune responses), accompanied by activation of complement cascade by circulating antibody-tumor-antigen complexes [62, 63, 64 and 65].

All constitutional manifestations are usually reversible and completely resolved after complete surgical excision of tumor tissue.

### ***Infection***

Although often present with constitutional symptoms mimicking infective endocarditis, CMs are rarely actually infected.

In most of the cases isolated pathogens belonged to the Streptococcus species (*Streptococcus viridans*, *Streptococcus oralis*, and *Streptococcus mutans*). There are also reports of CM infected by other bacterial and fungal pathogens (*Enterococcus faecalis*, *Staphylococcus lugdunensis*, *Gemella morbillorum*, *Porphyromonas asaccharolytica*, *Candida albicans* and *Histoplasma capsulatum*) [65, 66, 67, 68, 69, 70, 71, 72 and 73].

Superimposed infective processes render tumor tissue even more friable and prone to systemic embolization.

### ***Recurrence and malignant potential of cardiac myxoma***

Complete surgical resection of tumor tissue is currently the only treatment of choice for effective therapy of cardiac myxoma.

The surgery should be performed as soon as possible due to prominent danger of embolism, obstruction and sudden death registered in approximately 8-10% of patients awaiting operation [74].

Recurrence after surgical resection of primary lesions has been observed in 1-4% of sporadic and 12-22% of familial (CNC) cases [2, 3 and 12].

Secondary lesions usually occur as unifocal resurgence at or near the site of primary tumor or they can emerge in previously healthy endocardium of the original or other cardiac chambers [75 and 76]. Multiple secondary lesions are also recorded (even when the original tumor was single) as well as metastatic extracardiac lesions in arteries, bones, brain and other soft tissues [77, 78, 79, 80 and 81].

Re-recurrence is unlikely and only a few cases have been recorded [82, 83 and 4].

Secondary lesions are more common in younger patients. They may occur within just a few months or up to several years after the initial resection (average 4 years). There is also a report of patient in whom secondary lesion was diagnosed 20 years after the removal of primary tumor [85].

The explanation for the intracardiac recurrence can be found in familiar predisposition, unrecognized multicentric origin of primary lesion, incomplete resection or intraoperative dissemination of tumor cells and the *de novo* proliferation of the pretumor or reserve cells reportedly present in the endocardium [2, 4 and 86].

Although repeatedly hypothesized since the first reported case of recurrent left-sided atrial myxoma in 1967, relationship of local intracardial recurrence with the adequacy of the surgical resection is quite controversial. It appears that recurrent tumors often do not resurge at the site of the original lesion even in the cases when complete excision has not been done [8]. Furthermore, number of large series of postsurgical follow-up reported no recurrent tumors.

Biological characteristics of the CM assessed through expression analysis of tumor tissues and detection of existing DNA mutations, rather than their overall pathohistological presentation, may be the most reliable factor for recurrence prediction.

The recurrence is highest among patients with CNC and all of them carry some sort of DNA mutations [4 and 8].

Abnormal DNA has also been recorded in 20% of sporadic CM and their recurrence rate (12-40%) was significantly higher compared to overall sporadic cases (1, 2, 3, 4 and 8).

Additional problem is presented with sporadic CM recurring in distant regions of the body, especially the ones with apparently malignant nature [4, 87, 88, 89 and 90].

As a plausible explanation for their metastatic recurrence in local and distant extracardiac regions some authors propose survival of neoplastic cells in disseminated tissue fragments or detached overlaying thrombi and their further growth at the site of embolization. Other authors, taking into account the benign histology of primary lesion, consider that mechanism as quite unconvincing [4].

There are also reports of recurrent lesions with more aggressive histology and significantly faster cell proliferation, suggesting successive malignant alteration of benign tumors in their new settings [4, 87, 88, 89 and 90].

A more likely explanation may be that sporadic CM that later metastasize was a mistaken identity of the malignant primary tumor like myxosarcoma, fibromyxosarcoma, chondrosarcoma or malignant fibrous histiocytoma [91, 92, 93 and 94].

Only a few molecular studies have been conducted to evaluate proliferative activity and metastatic potential of CM or to establish expression pattern and polymorphism of proto-oncogenes and tumor suppressor genes in their cells. According to them CM represent a weekly proliferative lesion with little metastatic potential. All proto-oncogenes and tumor suppressors gene studied in those experiments showed either no (e.g. p53) or minor expressional modulation and/or genetic abnormalities of investigated proto-oncogene and tumor suppressor genes (Rb1, Bcl-2,) [95 and 96].

Expression microarray analysis reported in 2004 showed more than 10-fold higher expression of melanoma inhibitory activity (MIA) in sporadic CMs compared to normal tissues controls [97].

MIA has an important role in malignant transformation, growth, invasion, dissemination, drug resistance and immunoreactivity of malignant melanomas.

MIA was also reported in ovarian, renal and head/neck carcinomas as well as in many patients with breast and advanced gastrointestinal carcinomas. It seems that in number of patients increased MIA expression also correlates with progression to systemic metastasis and a poor prognosis [98].

Interestingly, an isoform of S100 protein, another molecular marker found in sporadic cardiac myxoma, has been recently established as a second potential prognostic marker for patients with malignant melanomas [97]. Altered expression of S100 family members is seen in many cancers including breast, lung, bladder and kidney as well as thyroid, gastric, prostate and oral cancers [99].

However, establishment of possible role (if any) of MIA and S100 (immunohistological studies report positive S100 staining without precise determination of protein isoforms) in tumor growth, recurrence and exact nature of their extracardiac dissemination or malignant potential of sporadic CMs requires further studies.

Another factor that may explain or at least contribute to the malignant potential of otherwise histologically benign CM and/or the remote metastatic growth of embolized material (through direct stimulation of cell growth and/or induction of angiogenesis) is autocrine expression of VEGF and PDGF and its receptors (VGER1/Flt-1, VGFR2/KDR/Flk-1 and PDGFR  $\alpha/\beta$ ) detected in the cytoplasm of tumor cells [100, 101, 102 and 103].

Inflammatory cytokine (IL6, IL8) abundantly secreted by neoplastic tissue also have the potential to enhance CM vascularisation and migration and proliferation of their cells. Important angiogenic signals may also be provided through action of monocyte chemotactic protein-1 (MCP-1) and thymidine phosphorilase (TP) whose expression is also detected in neoplastic myxoma cells (104).

Due to the rarity (number of cases presented in the same institution) of cases with seemingly malignant nature and lack of experimental data, sequential malignant transformation of primary benign tumor cells and the existence of malignant subpopulations of CMs is still a matter of high controversy.

### ***Macroscopy***

Cardiac myxomas have heterogeneous histomorphology that varies with their location, clinical presentation and age and gender of affected patient [1, 2, 3 and 12].

They show variegated color, consistency and size with pale gray, white, yellow or brown, somewhat bosselated surface appearance and fibrous, gelatinous or myxoid structure admixed with superficial thrombi or hemorrhagic dark brown or red areas [1, 2, and 3].

Their size range, from just a few millimeters up to 15 cm in diameter (average 5-6 cm), with corresponding weight from two to more than 250g [1, 2, 3 and 12].

Majority of CM are polypoid, pedunculated (rarely sessile), round or ovoid tumors with smooth, glistening or slightly lobulated surface, and a short broad base [1, 2, 3 and 12].

Polypoid myxomas are usually compact, solid tumors with little tendency to spontaneous fragmentation.

The less common are papillary myxoma with a multiple thinner or thicker villous, finger-like extensions and soft, gelatinous structure very prone to fragmentation, erosion and embolisation. [1, 2, 3 and 12].

As another source of embolisation papillary myxomas often contain superficial blood clots and hemorrhagic foci embedded among their villous extension. Contrary, in polypoid tumors hemorrhagic foci are more frequently internalize [105].

It seems that the occurrence of these two types of CM is not merely a matter of random events during their development.

Papillary myxomas show significantly higher expression and activity of specific matrix-metalloproteinases (MMP-MT1, MMP-2 and MMP-9), enzymes involved in extracellular matrix (ECM) remodeling [106].

Their increased activity leads to amplified degradation of ECM, distorts the balance between synthetic and degradation processes that changes mechanical properties of tumor tissue and most probably facilitates its fragmentation, erosion and embolization by hemodynamic forces. Furthermore, initial findings suggest that increased expression of MUC5AC gene within tumor tissue of sporadic myxoma may correlate with lower risk of embolization [107 and 108].

The two morphological subtypes of CM may also be differentiated by their usual clinical presentation [109].

Obstructive heart failure is usually associated with solid, polypoid tumors while neurologic and other embolic events represent the most common clinical feature of fragile papillary myxoma. Les frequently, turbulent blood flow can also lead to dissemination of emboli from the surface of solid myxoma [55 and 105].

### ***Histopathology***

CM are mainly composed of stellate, fusiform or elongated, polygonal (lepidic) cell incorporated into amorphous myxoid matrix [1, 2, 6, 12 and 110]. The cells usually occur solitary or arrange in small parallel clusters, short syncytial cords and vasiforme rings (multilayered circular structures around thin-wall blood vessels /perivascular cuffing/) and tubular structures [1, 2, 3, 105, 111, and 112].

Neoplastic tumor cells contain oval, round or elongated nuclei with finely dispersed or vesicular chromatin structure, a small amount of highly eosinophilic cytoplasm and indistinct



cell membrane. Syncytial multinuclear (3-9 nuclei) giant tumour cells can also be seen. Mitoses are rarely observed [1, 2, 6, 12 and 110].

Other cellular elements include variable number of blood cells (lymphocyte, erythrocyte, macrophage, mast cell, dendritic cell, plasma cell), histiocytes, fibroblasts and smooth muscle cells [1, 2 and 3].

Atypical cellular and extracellular structures like well-defined columnar epithelium occasionally forming glandular structure (~2% of tumors; may be confused for metastatic adenocarcinomas), foci of extramedullary hematopoiesis (~7%), chondrocytes and osteoblasts, calcification (~ 10%; more frequent in right atrial myxoma) and even metaplastic bone formation as well as cysts and degenerated collagen fibers encrusted with iron and calcium (Gamma-Gandy bodies) may also be encountered. Thymic rests have also been observed [113, 114, and 115].

The number of cells is highly variable, not only among tumors from different patients but also in different regions of the same specimen. The same heterogeneity applies for extracellular matrix and tumor vasculature.

The surrounding weakly basophilic, afibrillar myxoid matrix is abundant in proteoglycans made of variable protein core covalently linked to one or multiple glycosaminoglycan (GAG) chains. More than 90% of GAGs are composed of chondroitin-6-sulfate, hyaluronic acid and chondroitin-4-sulfate [1, 2 and 116].

Matrix contains variable amounts of elastin, fibrinogen, fibrin and collagen fibers and hemosiderin deposits scattered within the stroma or within histiocytes and myxoma cells [1, 2 and 3].

While only a subset of CM has macroscopically visible areas of hemorrhage, dispersed microscopic foci of recent and organizing hemorrhage is almost universal feature of all surgically resected tumors, also found in more than a half of the specimens obtained at

autopsy.<sup>41</sup> Hemosiderin deposits and Gamna-Gandy bodies are also microscopic remnants of old episodes of hemorrhage [1 and 2].

Myxomas are mostly perfused with thin walled blood vessels without pericytes while vessels with thicker muscle walls predominate at the base and in their stalk [1 and 2].

Tumor surface is covered with a single layer of flattened endothelial cells (multilayering may also be present) that form small vascular spaces or invaginations into the tumor stroma. Single layer of lepidic tumor cells may cover some surface regions [1, 2 and 3].

Superficial regions of CM tissue show a prominent collagenisation. That feature is absent in papillary tumor types.

### ***Histogenesis***

Controversy about the exact nature and histogenesis of CM exists since they were first described.

Initially it was believed that they originate from organized intracardial thrombi [1, 2, 117 and 118].

Based on their prevailing location, histological organization, observed chromosomal abnormalities and DNA ploidy pattern it was soon recognized that they represent a distinct clinical entity [1, 2, 119 and 120].

As the cardiac surgery and removal of neoplasms was gradually becoming a quite common procedure it was even more important to establish the criteria for their differentiation from other cardiac neoplasms, and to understand their histogenesis.

However, the infrequency of CM and heterogeneous results from various phenotypic characterization studies has limited the potential and there is still no clear conclusion about their exact origin.

The presence of heterologous histological presentation still suggests to some authors that CM may be the consequence of some reactive (traumatic) or hamartomatous processes [95 and 121].

### ***Viral origin***

Li *et al* in 2003 reported the presence of viral antigens and genetic material of herpes simplex virus type 1 (HSV1) in 70% of surgically removed sporadic CM [122].

Although their research was conducted on a relatively small number of cases (n=17), due to the prevalence of myxoma with viral DNA (8/17) and HSV1 antigens (12/17) they suggested that at least some CM may result from a chronic inflammatory lesion of endocardial tissue induced by viral infection[122].

Their hypothesis was further based on similar constitutional manifestations and abnormal laboratory findings present both in patients with CM and those with infective diseases [122].

Furthermore, multinucleated giant cells present in CM correspond to giant cells found in tissues infected with HSV1, and absence of mitosis and MIB1/Ki67 expression in these cells suggests their formation through fusion of mononuclear cells, mediated by HSV1 glycoproteins rather than proliferation [123 and 124]. Also, cuffing of the thin-walled blood vessels and microscopic hemorrhage present within myxoma tissue as well as fibrin deposition in tumor matrix histologically resembles lesions of solid tissues infected by HSV1 [1,2,3 and 110]. IL6 and VEGF expression found in cells infected by HV1 is also established in CM and HSV1 replication in neurons of satellite cells of cardiac ganglia and endocardium also correlates with hypothesized neuroendocrine origin of CM [61, 62, 63, 64, 65, 101, 102, 103, 125, 126 and 127].

However, recent immunohistological studies conducted on a series of 70 patients with cardiac myxoma showed no association between HSV1 and occurrence of CMs [128].

Based on numerous immunohistological, ultrastructural and in vitro studies, most authors now believe that CM represents true benign neoplasms developed from sub-endothelial vasoformative reserve cells or primitive stem cells (multipotential mesenchymal cells) residing in the fossae ovalis and surrounding endocardium [1, 2 and 3].

It is assumed that these multipotential mesenchymal cells exist in the endocardium as remnants of septation processes of the embryonic heart.

### ***Prichard's structures***

Exploring the potential presence of precursor cells in the fossa ovalis and surrounding endocardium, in 1951 Prichard detected microscopic intracavitary and subendothelial structures (lacunas of capillary size lined by plump endothelial cells) with predilection for interatrial septum. For a time these "Prichard's structures" were correlated with histogenesis of CM [129].

In order to confirm the existence of Prichard's structures and to clarify their possible role in histogenesis of CM, Acebo *et al*, and later Val-Bernal *et al* conducted a prospective, immunohistological studies of a large number (n<sub>1</sub>=100, n<sub>2</sub>=101) of interatrial septa [130 and 131].

Although they found structures similar to ones described by Prichard there were positive only for known markers of mature endothelial cells (vimentin+, CD31+, CD34+, thrombomodulin+, ckit/CD117 -; VEGFR2 -; Ki67 -) and negative for markers of embryonic/fetal endothelium and other primitive mesenchymal cells in various stages of differentiation (alpha-smooth muscle actin  $\alpha$ SMA, S100 protein, calretinin CALB2, and c-kit/CD117).

Prichard's structures were not found in the atrial tissue from the bases of any of the conventional cardiac myxomas and the patients with these structures were, on average 10 years older than the patients without them.

Based on their results they stated that Prichard's structure emerge as age-related senescence of endothelial cells with no apparent relation to histogenesis of CM.

Val-Bern *et al* hypothesized that Prichard's structures emerge by infolding of terminally differentiated endothelial cells lining the endocardium as an irritational, excessive growth response to altered or turbulent blood flow, and in fact represent the cardiac version of the cutaneous senile angioma [132 and 133].

### ***Multipotential mesenchimal cells***

Immunohistochemical characterization of CM conducted so far resulted with heterogeneous phenotype, with neoplastic cells expressing various antigens (often within the same tumor) specific for different cell lineages including epithelial, endothelial, myogenic, myofibroblasts or neuroendocrine differentiation markers [1, 4, 12 and 110].

Pluripotency of these primitive mesenchimal cells, heterogeneous phenotype of myxoma cells and different approaches in their morphological and immunohistochemical characterization are main reasons for somewhat conflicting hypotheses about the histogenesis of CM.

### ***Cardiomiogenic differentiation lineage***

To clarify the inconclusive data regarding the histogenesis of CM Kodama *et al* examined the expression pattern of several cardiogenic transcription factors (Nkx2.5/Csx, GATA-4, MEF2 and eHAND) specific to phenotype of primitive cardiomyocytes [134].

Expression of these transcription factors starts early in various regions of precardiac mesoderm and primitive heart tube but is later restricted to specific heart structure and maintained at higher levels in cardiac muscle cells of postnatal heart throughout the life.

Using the immunohistochemical and RT-PCR approach Kodama *et al* confirmed their variable expression activity (slightly-to-intensely positive) in the nucleus and cytoplasm of tumor cells in all myxoma specimens (n=5). Furthermore, in situ hybridization for Nkx2.5/Csx was also positive for all myxoma while nested-PCR approach confirmed the expression of Nkx2.5/Csx in all and eHAND in 50% of cases.

Contrary, myosin light chain kinase v2 (MLC-2v) specific for terminally differentiated cardiac muscle cells was RT-PCR negative.

Based on those results Kodama *et al* concluded that expression of selected transcription factors specific to phenotype of primitive cardiomyocytes clearly suggests development of CM from multipotential mesenchymal progenitors with a cardiomiogenic lineage.

Hypothesis that CM originate from remnants of primitive multipotential cardiogenic cells present in mature heart was further examined by Orlandi *et al* [135].

Their findings suggest that neoplastic CM cells exhibit some phenotypic markers of embryonic endothelial-to-mesenchymal transformation (EMT) that precedes terminal differentiation of endocardial cushions and/or markers of primitive cardiac mesenchymal differentiation.

Their concept is supported by morphological comparison of CM with embryonic endocardial cushions that has revealed substantial similarities between the endothelial lining cells, the cells within the cushion tissue and lepidic cells of CM [2].

Even the prevailing location of CM near the fossa ovalis on the left side of the interatrial septum could suggest their close relationship with fibrous cardiac structures and embryonic endocardial cushions from which they arise.

During the process of cardiac embryogenesis primordial endocardial endothelial cells involved in the EMT transformation (induced by signals from primitive myocardium) gradually change their expression pattern, lose apical/basolateral polarity and reduce the number of intercellular bonds.

They break down the basement membrane (delamination), separate themselves from the endocardium, proliferate and gradually obtain migratory phenotype of mesenchimal cells. Concomitantly they invade the cardiac jelly (promotes the initiation of EMT transformation) and later participate in the formation of endocardial cushions.

As a part of gradual alteration in their expression pattern, concomitantly with their EMT transformation embryonic endocardial endothelial cells progressively express mesenchimal markers (e.g.  $\alpha$ -SMA), and shut of the expression of endothelial cell markers [133].

Using the immunohistochemical and RT-PCR approach Orlandi *et al* confirmed the expression of  $\alpha$ -SMA protein and/or transcript in majority (83.3%) of analyzed left-sided atrial myxoma (n = 30). They also detected focal expression of  $\alpha$ -CA ( $\alpha$ -cardiac actin) in only 10% of specimens while expressions  $\alpha$ -SKA ( $\alpha$ -skeletal actin) was negative in all samples.

Since expression of  $\alpha$ -SMA is transiently expressed in cardiomyocytes during the early period of fetal development while  $\alpha$ -CA and  $\alpha$ -SKA are co-expressed in mature myocardium as prevailing isoforms, observed pattern of sarcomeric actin expression in lepidic cells of CM indicates their morphological similarity to primitive cardiac progenitors or primordial cardiac stem cells [136 and 137].

Furthermore,  $\alpha$ -CA is a major isoform of sarcomeric actin present in the cardiomyocytes of the 20 weeks old fetal heart (heart septation is completed and the heart poses all morphological characteristic of mature organ) with uniform expression throughout the myocardium.

Beside the heterogeneous and mutually exclusive expression of CD34 and  $\alpha$ -SMA (primitive endothelial and myocytic markers, respectively) in different cells of atrial myxoma they detected a small number of neoplastic cells that co-express CD34 and  $\alpha$ -SMA.

The intermediate phenotype of CD34/ $\alpha$ -SMA positive cells supports the concept of primordial differentiation of myxoma from early cardiac precursor cells. That is further supported by detection of focal co-expression of Flt-1 and Flk-1 (early stem marker for endothelial cell precursors) [138].

To further confirm their hypothesis Orlandi *et al* have performed RT-PCR analysis of several early cardiac differentiation markers and regulators (Notch1, WT1, NFATc1, Sox9, ErbB3, SMAD6, MMP1, MMP2, CALB2, TIMP1).

They detected the presence of CALB2, MMP2, TIMP-1 and Sox9 in all examined myxoma specimens (n = 8). Notch1 was observed in 87.5%, and NFATc1 in 37.5% of samples. Expression of ErbB3 and WT1 was absent in all specimens.

Furthermore, more than 50% of samples were positive for MMP1 while SMAD6 was detected in only two cases.

Their results support stated hypothesis and substantially correlates with the role of analyzed genes during EMT transformation of endocardial cells.

However, based on those results Orlandi *et al* were not able to conclude whether the CM derives from embryonic remnants of cardiac cushions or primitive multipotential mesenchimal cells present in adult heart or even from ectopic *de novo* re-expression of early cardiomiogenic phenotype in adult cardiac cells.

### ***Neuroendocrine differentiation***

Detection of vasointestinal peptide (VIP) in some CM and their co-presentation with other features of CNC syndrome (lentiginosis, cutaneous neurofibromas, blue naevi,



phaeochromocytomas and endocrine tumors in testis and pituitary gland) led Krikler *et al* to investigate their possible neuroendocrine origin [139].

They immunohistologically examined expression of markers specific for neuroendocrine (protein gene product 9.5/PP9.5, neuron specific enolase/NSE, S100, synaptophysin p38/SYP), endothelial (von Willebrand factor VWF/FVIII, CD34) and smooth muscle cells ( $\alpha$ -SMA) in 24 CM (3 were CNC) samples.

Tumor cells (single stromal cells or cells in outermost layer of concentric vessel-like clusters) were positive for PP9.5 in 75% and S100 in 66.7% of cases while 50% of specimens were positive for NSE and 29.16% for SYP.

Myxomas positive for NSE were also positive for S100 and PP9.5 while 58.33% of NSE positive tumors co-expressed SYP protein.

Furthermore, surface of myxoma positive for PP9.5 was partially covered with PP9.5+ cells.

Only one CNC sample was positive (stromal cells) for PP9.5 and NSE.

Cells positive for  $\alpha$ -SMA were mainly found in the thick-walled blood vessels at the tumor base. Solid masses of proliferating smooth muscle cells unrelated to blood vessels were also found.

Observed expression pattern of neuroendocrine markers and distribution of VWF/FVIII, CD34 (inner layer immediately surrounding lumens of vessel-like structures), and  $\alpha$ -SMA led Krikler *et al* to suggest possible neuroendocrine origin of cardiac myxoma.

Hypothesis of myxoma origin from multipotent mesenchymal cells capable of neural (and endothelial) differentiation was also suggested by Pucci *et al* [112].

They immunohistochemically investigated 53 sporadic myxomas for neuroendocrine (PP9.5, S100, NSE) endothelial (FVIII/VWF, CD34, CD31, ulex europeus agglutinin/UEA-1), smooth muscle ( $\alpha$ -SMA) and epithelial cell markers (carcinoembryonic antigen/CEA) as well

as cytokeratin CK 9p, atrial natriuretic peptide (ANP), VIP, calcitonin gene-related peptide (CGRP) and chromogranin (ChrA, ChrB).

All neuroendocrine markers were detected in 57% of tumors while the most common co-expression was found for PP9.5 and S100 (85%). Positive PP9.5 staining was detected in 94%, S100 in 89% and NSE in 57% of cases.

Immunoreactivity for  $\alpha$ -SMA was noticed only in the media of blood vessels and in smooth muscle like cell groups interspersed in stroma (distinct from stromal cells).

Expression of FVIII/VWF was observed in the inner endothelial layer of vascular-like aggregates, vessel endothelium and in stromal (23%) tumor cells.

Another endothelial marker, CD31 was expressed by vessels endothelium of all cases (n = 6), while the stromal cells were also positive for CD34 (4/6) and UEA-1 (2/6) antigens.

All stromal cells were negative for ChrA, ChrB, ANP, CGRP and VIP antibodies.

Positive staining for CK 9p, CEA, S100, and NSE were also found in glandular epithelial structures (2 cases). Since their expression pattern overlaps with the pattern of endocrine cells from human gut epithelium, Pucci *et al* suggested that these glandular elements may represent rests of embryonic foregut.

Furthermore, positive reaction for S100 and NSE was also observed in chondroid-appearing tumor areas.

Neuroendocrine origin of CMs was further supported with findings of Teraccianno *et al* [140]. They detected strong and diffuse immunohistochemical staining of CALB2 (calretinin/calbindin 2 protein normally detected in the cells of the central and peripheral neural tissue) in the cytoplasm and nucleus of neoplastic cells of all examined (n=24) sporadic CM cases [141].

Furthermore, expression of CALB2 by ganglion cells in the fetal heart also supports endocardial sensory nerve origin of cardiac myxoma.

Hypothetical origin of CM from primitive mesenchymal cells is also supported by findings of Sakamoto *et al* [142].

Using the enzyme-linked immunosorbent assay (ELISA) they detected increased (compared to human umbilical vein endothelial /HUVE/ cells) constitutive protein expression of Endothelin-1 (ET-1) and its precursor big ET-1 in cell lines established from CM tissue of two patients. They also detected increased expression of IL6, IL8, CXCL1 and growth-related oncogene  $\alpha$  and no expression of stem cell factor, granulocyte colony-stimulating factor and hepatocyte growth factor in myxoma (and HUVE) cell lines.

The obtained results prompt them to support the hypothesis that CM originates from mesenchymal cells capable of endothelial differentiation.

CXCL1 chemokine also serves as autocrine factor for melanoma cells and as neutrophil chemoattractant and angiogenic factor as well. Overexpression of growth related oncogene  $\alpha$  promotes tumor growth and metastasis and its presence in cardiac myxoma, together with CXCL1, may further explain the reported malignant potential of some CM.

Recently, after histological and electron microscopy examination of 168 cases, Rogov *et al* had revealed signs of embryonal endothelium capable of vasoformation and glycoprotein/glycosaminoglycan synthesis in neoplastic cells of CM [143].

According to them CM is a true benign dysontogenetic tumor originating from embryonal endothelium but the term myxoma does not faithfully reflect its true morphological entity so they suggested the name embryonal endocardial endothelioma [143].

Johansson and Curschellas *et al* suggest myxoma origin from embryonic cell remnants (based on expression of F8, Des, VIM, cytokeratins, S100, LU5, CAM5.2 and NSE) while Farrell *et al* and Lindner *et al* (based on observed expression of CD34, CD31, CEA and CA19.9) suggested their endodermal origin [144, 145, 146 and 147].

### ***Bioinformatic analysis***

Bioinformatic analysis [performed with the aid of the online DAVID database- The Database for Annotation, Visualization and Integrated Discovery software v6.7, <http://david.abcc.ncifcrf.gov/>] of currently known markers and antigens (whose data are publicly available in corresponding literature databases) expressed by CM (see *Table 1.*) and grouped by functional annotation clustering (default parameters) reveals 34 key protein markers possibly related to histogenesis of CM and development of their heterogeneous cellular and structural components.

Due to the unspecific nature of investigated cell lineage markers this approach only confirms their overlapping functionality in various developmental processes such as: endothelial-to-mesenchymal transformation (NOTCH1, SOX9, NAFTC1), mesenchymal cell differentiation (NOTCH1, SOX9, EDN1, EDN3, NAFTC1), cell fate commitment (NOTCH1, SOX9, FGF2, KDR), regulation of cell development (NKX2.5, NOTCH1, TIMP2, EDN1, FGF2, MIB1, SPP1), heart development (GATA4, NKX2.5, NOTCH1, SOX9, ENG, EDN1, HAND1, MIB1, MYH10, NFATC1, ACTC1, PKP2), heart looping (GATA4, NKX2.5, ENG, HAND1, MIB1), muscle cell/tissue differentiation and development (ACTC1, NKX2.5, KRT19, RB1, ENG, HAND1, MYH10, PDGFRB, TNC) regulation of muscle development (GATA4, NKX2.5, NOTCH1, FGF1), neuron differentiation (CD44, NOTCH1, EDN3, FGFR1, IL6, MYH10, UCHL1/NSE, VEGFA), regulation of neuron differentiation (NKX2.5, NOTCH1, TIMP2, MIB1, SPP1), epithelium development (CD44, ENG, KDR, MIB1, VEGFA), ectoderm and epidermis development (NOTCH1, SOX9, KRT9, PDGFA), skeletal system development (SOX9, EDN1, FGFR1, MMP14, MMP2, MMP9, PDGFRA, PDGFRB, SPP1), ossification (MMP14, MMP2, SPP1), bone development (SOX9, MMP14, MMP2, SPP1) and angiogenesis (NOTCH1, ENG, EDN1, FGF2, FLT1, HAND1, IL8, KDR, MMP14, PDGFA, TYMP, VEGFA).

Key proteins expressed in CM are also involved in various overlapping signaling pathways (e.g. G protein coupled receptor signaling pathway, VEGFR signaling pathway, TGF $\beta$  receptor signaling pathway, MAPK signaling pathway, cytokine-cytokine receptor interaction and intracellular signaling cascade), cell cycle regulation and cell proliferation, apoptosis, cell adhesion and cell migration and metastasis (*Table 1*).

Gene functional classification of protein markers performed by the same program (DAVID database) reveals only three functionally related groups of gene/protein markers mainly related to: metalloproteinase involved in extracellular matrix remodeling (MMP1, MMP2, MMP3, MMP9, MMP14), cytoskeletal intermediary filaments (KRT7, KRT8, KRT9, KRT19, KRT20, DES, VIM) and tyrosine specific protein kinase related activities (FLT4, PDGFRB, FGFR1, FLT1, KDR).

Presented results of functional annotation gene clustering are in good agreement with the extensive bioinformatics study reported by Barh and Parida in 2009, which have found key CM protein markers to be involved in G protein signaling, heart development, angiogenesis, cell proliferation, cell adhesion, cell migration and metastasis [12].

After extensive literature search they grouped all molecular markers found in CM based on their expression pattern (expressed, down regulated up regulated and highly up regulated in malignant cases) and used them to construct protein-protein interaction maps [Osprey 1.0.1 software powered with human GRID database (<http://biodata.mshr.on.ca/osprey/servlet/Index>)], general disease pathway (using the data from Invitrogen, KEGG, BioCarta, Ambion, and Cell Signaling pathway database) and finally for the construction of final critical disease pathway responsible for cardiac myxoma development (obtained by the Cell Designer 4.0 beta software used for analysis of signaling networks, key nodes and up and down-stream target analysis).

Their results shows that common myxoma molecular markers interact with various other proteins involved in numerous biological processes such as tumorigenesis, heart development, and NOTCH and growth receptor signaling pathways.

Several disease pathways were found to interplay with each other with the main pathways including CCR2, FMOD-TGFB, S100A1-FGFR, NKX2.5-GATA4-SOX9-FGFR, HAND1-GATA4, and MUC1.

For example, some proteins involved in heart development (GATA4, SOX9, HIND1) interact with the signaling receptors by growth factors via KPNB. The NOTCH1 and several mitogenic pathways associated with them over the MUC1 whereas F8, THBD, FMOD, PLA2G2A, CCL2 and CCR2 interact with the rest of protein network via MMP2 protein.

At the certain point majority of these pathways were found to follow the growth receptor signaling pathway with CCR2, TGF- $\beta$ , MUC1, FGFR, EGFR, GATA4 and HAND1 identified as their key node elements and MYC, FOS and MMP9 as one of the common downstream targets.

All these key nodes, their regulators and upstream and downstream targets can be considered as potential drug targets that can be used to block the specific pathways responsible for CM development.

### ***Microarray analysis***

Performed bioinformatics analyses are expectedly biased by the short extent and type of antigens and protein markers investigated so far to phenotype CM, with most of them used just to decipher the specific (endothelial, cardiomyogenic or neuroendocrine) cell source of myxoma origin.

More reliable and extensive data for investigation of regulatory pathways activated in tumor cells, identification of the myxoma cell origin and better understanding of their various

clinical manifestations and reported malignant/metastatic potentials can be obtained through global gene expression analysis using either cDNA or other recently developed mRNA or protein and miRNA microarray platforms combined with laser capture technology (homogeneous self source) and confirmation of obtained data through qPCR or various immunohistological and blotting techniques.

So far only one global microarray study has been reported by Skamrov *et al* [97].

They performed the cDNA expression array analysis of seven sporadic CM cases comparing them with the expression profile of a selected group of physiologically normal tissues (blood, aorta, heart, brain, lung, spleen, kidney, liver, small intestine, skeletal muscle, pancreas, stomach, colon, bone marrow, and skin).

After extensive bioinformatics analysis (taking into account only the genes that have the same order of expression intensity in no more than two out of 15 used normal tissue controls) they ended up with the narrower sets of genes (out of 5200 analyzed) that can be potentially used as molecular markers of CM. The data were further confirmed by the gene specific RT-PCR analysis.

Obtained results clearly showed that CM could be viewed as standalone histological entity rather than a pathological modification of normal neighboring tissue.

Interestingly, according to applied criteria, antigenic markers commonly discussed in connection with CM (S100, CCL2/MCP1, CD34, FVIII/vWF and FVIII related protein, vimentin and laminin) were not selected as specific markers since they exhibit either high level of expression in number of used control tissues or high variability in examined CM samples.

Gene markers specific for endothelial, smooth muscle and blood cells did not showed dramatic variability suggesting that those cellular elements were present in more or less

constant proportions in examined CM cases while markers for normal cardiomyocytes showed relatively low and highly variable expression level.

The most prominent expression among selected mRNA markers steadily expressed in CM, was shown by MIA and PLA2G2A (more than 10 fold higher expressions compared to controls) and together with PLTP they also showed the highest specificity.

Interestingly both of them have proven role in progression of various malignant diseases. Contrary to the role of MIA in melanoma development, increased expression of PLA2G2A in human adenocarcinomas of the stomach substantially correlates with higher life expectancy and lower risk of metastasis.

Selected CM markers are involved in phospholipids metabolism (PLA2G2A, ANXA3, PLTP), regulation of cellular proliferation and inhibition of proteases involved in ECM remodeling (TIMP1, SLP1) while others function as modulators of ECM-cell interactions (MIA, SPP1, FMOD), transcription factors (SOX9) or intracellular calcium binding proteins (CALB2).

The presence of genes, specific for chondrocytes development (SOX9, MIA, SPP1) within the selected group of CM markers also indirectly supports their origin from primitive multipotential mesenchymal cells.

### ***Cytogenetics***

CM exhibits distinct aneuploid/tetraploid DNA pattern and karyotype heterogeneity even within the different areas of the same tumor [1, 2, 3 and 8].

Most of the reported cytogenetic data relate to patients with familial pattern of the disease (CNC) that exhibits various chromosomal abnormalities primarily involving chromosome 2, 12 and 17 [19, 20, 21, 148, 149, 150, 151, 152, 153 and 154].



Initial linkage gene analysis indicated the existence of two major CNC loci possibly harboring the myxoma susceptibility genes: CNC1 located at 17q22-24 and CNC2 located within the chromosome 2p16

The CNC1 susceptibility gene recently identified by two independent research groups is the PRKAR1A gene that encodes the R1 $\alpha$  regulatory subunit of cAMP dependent protein kinase A (PKA) involved in G protein receptor signaling pathways [20, 21 and 151 ].

Mutations in PRKAR1A were found in up to 80% of patients with familial history of CNC with only a few reports among sporadic myxoma cases [1, 20 and 21].

More recent large, transatlantic consortium study of 353 patients who carried a germline PRKAR1A mutation or were diagnosed with CNC and/or PPNAD disease revealed the existence 80 different PRKAR1A gene mutations. Among them 6 were missense mutations; 19 were nonsense mutation; 20 mutations involved splice-site; 33 caused a shift in reading frame (through insertion, deletion or another change involving a few base pairs) and two were characterized as deletions involving large intronic and exonic areas of the gene [155].

They were randomly distributed throughout the coding area, although mutations involving exons 2, 3, 5, 7 and 8 were more frequent. There were also two observed single mutational hot spots [c.491-492delTG in exon 5 and c.709-7del6 in intron 7] that occurred independently in several unrelated families of various ethnic backgrounds.

Certain mutations were more frequent and provided some genotype-phenotype correlation.

There were also observed gender differences among carriers of PRKAR1A mutations with PPNAD disease occurring earlier and more frequently in female patients, suggesting the existence of some tissue specific microenvironmental factors that may modify their tumorigenic potential.

Most PRKAR1A mutations (63) resulted with shortened, premature transcripts subjected to selective degradation through nonsense-mediated mRNA decay (NMD) that leads to R1 $\alpha$  haploinsufficiency and increased activity of PKA enzyme [21, 151, 156 and 157].

Only small subset of PRKAR1A mutations discovered so far are not subject to NMD mechanism, but they also result with dysfunctional protein and haploinsufficient CNC phenotype with increased PKA activity and more aggressive disease outcome [17 and 19].

Depending on the type of affected tissue downstream signaling effects of altered PKA action may include changes in metabolism, ion transport, hormone production and gene transcription with consequent alterations of cell cycle and induction of tumorigenesis but its exact role in tumorigenesis of cardiac myxoma has still to be established.

Rare individuals with a missense mutation (e.g. R74C), whose mRNA escapes NMD and is expressed as mature mutant protein seems to have the same haploinsufficient R1 $\alpha$  phenotype suggesting the possible existence of yet another molecular mechanisms not limited to inadequate control of PKA catalytic subunit but with different protein interactions (e.g. with PAP7 protein) responsible for increased tumorigenic potential of familial myxoma cases.

Recent genetic studies of a large family with CNC syndrome co-segregated with autosomal dominant TPS disorder [trismus-pseudocampodactility syndrome (TPS), also referred as Hecht-Beal syndrome] led to discovery of novel CNC gene locus mapped to chromosome 17p12-31. [155].

Sequence analysis performed at this locus showed a missense mutation (Arg674Gln) in the perinatal isoform of the myosin heavy chain gene MYH8.

Mechanism through which the mutation of MYH8 causes development of CM is still unknown. But since perinatal myosin is expressed in myofibroblasts it is speculated that MYH8 may act as a developmental switch that governs the loss of multipotent nature of primitive cardiomyogenic cells and their differentiation into a non-proliferative mature

myocardial tissue. It was hypothesized that mutations of perinatal myosin gene might promote the survival of multipotent progenitor cells in mature heart and provides a substrate for second tumorigenic genetic event (e.g. PRKAR1A mutation) that result in CM development. That hypothesis is in agreement with Knudson two-hit model of tumorigenesis but also with hypothetical origin of CM from residual sub-endocardial primitive stem cells.

Somatic alterations (amplifications, deletions) of CNC2 were observed in a large number of CNC patients but susceptibility gene has not been detected, and families initially assumed to have a major mutation in that region were subsequently linked to chromosome 17q24 [158].

The frequency of amplifications on 2p16 locus among CNC patients, including those with germline, inactivating PRKAR1A mutation suggests the presence of an oncogene whose increased activity may serve as another molecular event responsible for tumorigenesis in at least a subset of families with a CNC syndrome [21 and 153].

Previously mentioned consortium study has also revealed for the first time some phenotypic differences between patients with CNC1 defects and those with aberration in CCN2 locus but without PRKAR1A mutation. The later seemed to present later in life, were unlikely to have familial history of the disease and were less likely to develop myxomas, thyroid tumors, PMS and LCCSCT.

In contrast to genetic abnormalities in CNC patients no single gene mutations responsible for sporadic myxoma has been discovered.

Furthermore, cytogenetic analyses performed on sporadic CM have shown no role for 2p16 chromosome and only a limited involvement of structural rearrangements within 17q2 chromosomal region leading to conclusion that they are not genetically related to familial cases and that some other yet unknown mechanism must be responsible for their histogenesis [159]

However, detected clonal and more frequent non clonal structural aberrations such as microsatellite instability (e.g. ANK1, D2S105, D3S647, D7S479, D7S520, D17S250, D17S579, D17S855, D19S49), telomeric associations [e.g. tas(13;15)(p11.2;p11.2)], deletions (loss of the Y chromosome), translocations [e.g. translocation between chromosome 1 and 12 with a breakpoint at 12p12, addition (1)(q32) from unknown region etc.] and other rearrangements found in various sporadic CM cases particularly in chromosomal regions 12p1 and 17p1 suggest that these chromosomal rearrangements may contribute to tumorigenic pathways and progression of sporadic myxoma [17, 20, 160, 16, 162, 163 and 164].

However, whether there are any susceptible genes in these or neighboring regions that are related to the observed genetic instability has still to be elucidated.

### ***Epigenetics of cardiac myxoma- a road yet to be taken***

Reviewing the literature, and with the help of cardiovascular databases ([www.cardio.bjmu.edu.cn](http://www.cardio.bjmu.edu.cn)) Barh and Parida have also identify set of genes common to the processes of heart development and histogenesis of cardiac myxoma: NKX2.5/CSX, GATA4, HOX, HAND, MYOD, SOX4-6, S100 and TGF $\beta$ .

However, these genes are active at the early stage of embryonic heart development (mainly during EMT process) while majority of CM develops much later in middle age patients with very few cases registered in the early postnatal period of life[12 and 165].

That prompted Barh and Parida to speculate a possible role of epigenetic factors in reactivation and ectopic expression of these early embryonic heart genes that may contribute to CM development in much later, adult stages of life.

Epigenetic mechanisms are defined as inherited and/or acquired, mitotically and meiotically stable and reversible changes in phenotype that occur without the alterations in nucleotide

sequence of affected genes but control their temporal, spatial and quantitative expression patterns.

The important epigenetic mechanisms include DNA methylation of cytosine residues in CpG islands of gene regulatory elements, post-translational covalent modification of histone proteins or their replacement with alternative isoforms and/or non-coding RNAs (e.g. micro-miRNA) that are not translated into proteins but play essential roles in the post-transcriptional control of gene expression through complementary binding to their specific mRNA targets.

A sophisticated interplay between these epigenetic events changes chromatin conformation and patterns of gene expression (modulation, activation or inactivation) and is important in various developmental and disease processes.

For example, various miRNA molecules have already been associated with pathologic alteration of gene expression during progressive development of cardiovascular diseases such as hypertrophy of the heart. Furthermore recent *in vitro* experiments also showed that the methylation of genes and/or posttranslational epigenetic changes of their proteins (e.g. HDAC1, GATA4, H3 and H4) also play an important role during cardiomyogenic differentiation of multipotential mesenchymal stem cells in murine models [167, 168, 169 and 170].

Using a bioinformatics approach Barh and Parida selected a group of miRNA molecules (let-7, miR-125, miR-205, miR-214, miR-217 and miR-296) that may target majority of previously mentioned key molecular markers and potentially influence the entire critical disease pathway/s responsible for myxoma histogenesis [12].

However, until now there is no reported data neither for miRNA gene expression nor for other epigenetic factors possibly involved in histogenesis of CM and the precise role of these epigenetic modulators and putative therapeutic molecules has still to be experimentally elucidated.

### ***Conclusion and further directions***

Despite so many immunohistological and other studies the cellular origin of CMs is still controversial. The phenotypic expressions of tumors that originate from multipotential mesenchymal cells is often variable and maybe misleading, revealing just one feature of tumor tissue, which does not necessarily reflect the true origin of their cells or the main signaling and regulatory pathways responsible for their molecular pathogenesis.

Furthermore, all markers studied in CM tissue are not exclusively specific for endothelial, cardiogenic or neuroendocrine differentiation lineage and could be variably expressed in number of other cell types or ectopically re-expressed in adult tissue due to some kind of epigenetic deregulation.

Due to the rarity of the cardiac myxoma (small number of cases in one institution) and lack of comprehensive experimental data particularly concerning the recurrent cases, myxoma with metastatic dissemination and secondary lesions with malignant nature, more extensive multi-institutional approach involving the application of recently developed technology is crucial for better understanding of their biological background, molecular pathogenesis and for elucidation of their cell(s) of origin.

Advent of omics-technology with its unprecedented potential for concomitant investigation of complete cell transcriptome (on mRNA and miRNA level) in normal and pathological conditions, bioinformatic analysis of obtained data and their comparisons with the data from epigenetic and proteomic approaches enables a comprehensive investigation of entire CMs signaling networks and regulatory pathways and elucidation of their key molecular markers and their potential as drug targets.

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